PROPIONIC AND METHYLMALONIC ACIDEMIAS: A HISTORICAL REVIEW

by

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ABSTRACT

Methylmalonic and propionic acidemia are two organic acidemias. These inborn errors of amino acid metabolism have been studied extensively for decades. Through clinical and scientific study, researchers have been able to elucidate the biochemistry and cell biology that underlies these two diseases. This review gives a comprehensive overview of the history of the disease, as well as a scientific description of the related enzymes, cofactors, and genetic basis for disease. In conclusion, clinical manifestations and treatments are described, and recommendations for further study are provided.

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TABLE OF CONTENTS

INTRODUCTION

Branch chain amino acid metabolism has been a topic of study for researchers for decades. In studying branch chain amino acid metabolism, scientists have found many inborn errors of metabolism that cause disease. In examining both the pathways and the disease states, a full picture of the biochemistry behind branch chain amino acid metabolism can be elucidated.

Methylmalonic acidemia and propionic acidemia are two diseases that occur as a result of inborn errors of branch chain amino acid metabolism. Propionic acidemia was first described by Childs[1] in 1961, and was originally called ketotic hyperglycinemia. With continued research, it was discovered that the disease was an error of propionate metabolism that often, but not always, had ketosis and hyperglycinemia as clinical features. The next year, Cox and White[2] described methylmalonic acidemia as a metabolic error that manifests in some patients when serum cobalamin (B12) levels are low. Cox and White also showed that cobalamin administration would alleviate methymalonic acid levels in a single patient. Today it is understood that this patient did not have classical methylmalonic acidemia where the inborn error is found in methylmalonyl-CoA mutase, rather, he had a defect in cobalamin synthesis. Much work has been done in the last decades to understand the biochemical, genetic, and clinical components of these two diseases.

BIOCHEMISTRY

Propionate metabolism has been elucidated by studying animal and human tissues, primarily liver and kidney. In 1955, Lardy[3] showed that propionate is converted to succinate by enzymes in liver mitochondria in a range of animals. That same year, it was also reported by two groups that methylmalonate is an intermediate in propionate metabolism.[4,5] Additional studies confirmed that methylmalonyl-CoA was converted to succinyl-CoA.[6] Two years later, Flavin's group described a more complete sequence of propionate metabolism that included appropriate CoA esters.[7]

Methylmalonate as an intermediate was further shown to have two isomeric forms. In 1964[8], it was shown that the "D" form of methylmalonyl-CoA was formed directly from propionyl-CoA carboxylase. However, two years later, Sprecher[9] reported that methylmalonyl-CoA must be converted to its L-isoform in order to be converted to succinyl-CoA by methylmalonyl-CoA mutase. Meanwhile, Mazumder had isolated and purified methylmalonyl-CoA racemase from sheep liver, and had also determined that the racemization occurred by an α-hydrogen atom shift.[10] Thus, the components of the pathway were completely described.

Propionyl-CoA → D-methylmalonyl-CoA → L-methylmalonyl-CoA → Succinyl-CoA → Succinate

PROPIONATE SOURCES

Propionate is found in small quantities in the human body. It is an important part of amino acid metabolism, and is part of a pathway that eventually feeds into the citric acid cycle via succinyl-CoA. Propionate comes from three important sources in the body: anaerobic gut bacteria, branch chain amino acid metabolism, and β-oxidation of odd-numbered long chain fatty acids. In methylmalonic acidemia and propionic acidemia patients, the proportion of propionate generated from each source is somewhere around 50% from amino acids, and either 20%/30% bacteria/fatty acids[11] or 25%/25% bacteria/fatty acids.[12]

Childs[1] first described the amino acids that may play a role in producing propionyl-CoA. His studies implicated leucine, and to a lesser degree isoleucine, valine, and threonine. For methylmalonic acidemia, Rosenberg[26] implicated isoleucine and valine as disease causing. Interestingly it was 1975 before the full biochemical pathway was determined that showed that propionate was an intermediate of valine metabolism.[13, 14] It is now known definitively that isoleucine and valine metabolism are the leading amino acids that contribute to disease via propionyl-CoA formation.

ENZYMES

During the period that the biochemical pathways were being elucidated, the enzymes responsible for the reactions within the pathways were also being discovered, isolated, and purified.

Propionyl-CoA carboxylase was first described by Tietz[15] in 1959, where he described the mechanism of this enzyme, which included performing both CO_2 activation and carboxylation activites. Later, propionyl-CoA carboxylase was first crystallized in pig's heart and also shown to bind biotin.[16] Studies crystallizing the enzyme from bovine kidney[17] and human liver confirmed this.[18, 19] Propionyl-CoA carboxylase is composed of 2 non-identical subunit protomers[17], the larger of which binds biotin.[15, 17] The human enzyme is 750-800 kDalton in its native form,[20] and the mass of each subunit is 72 kDalton and 56 kDalton for the α and β subunits, respectively.[19] In conjunction with this data, it was also shown that the homologous enzyme in the bacterium *Mycobacterium smegmatis* has a $(\alpha\beta)_6$ configuration.[21] All of this evidence points to an $(\alpha\beta)_6$ configuration for the human enzyme as well.

The CO_2 fixation and the carboxylase activity of propionyl-CoA carboxylase happen in tandem. ATP is required and potassium[22] stimulates the first step which is forming a carboxybiotin-apoenzyme complex. The resulting complex reacts directly with the biotin in order to transfer the carboxyl group, forming methylmalonyl-CoA.[23] The biotin is directly linked to a well-defined biotin binding site on the α-subunit.[24]

Methylmalonyl-CoA mutase was described in 1959, as an enzyme analogous to β-methylaspartate in bacteria.[25] It was also shown to require B12 (in the form

adenosylcobalamin) at this time.[26] Isolation and purification of methylmalonyl-CoA mutase from human placenta showed that it is a dimmer composed of identical subunits.[27] Methylmalonyl-CoA mutase is covalently bonded to B12, which is a fact confirmed by isolation of methylmalonyl-CoA mutase from human liver.[28] It was demonstrated that activity of the enzyme was stimulated by anions, and inhibited by cations. In addition, hydroxocobalamin, a form of cobalamin, was found to be an irreversible and competitive inhibitor of the enzyme.[29] The enzyme is a homodimer with the molecular mass of 145 kDaltons, and each subunit binds one cobalamin. This is in contrast to the methylmalonyl-CoA mutase found in *Propionibacterium shermanii*, which is made of 2 non-identical subunits, where the α-subunit binds adenosylcobalamin. The human form of the enzyme appears to have unequal binding sites, as it has been shown to have complex kinetics.[29]

The mechanism by which methylmalonic-CoA mutase works is by an intramolecular shift of the CoA carboxyl group.[30, 31] This shift occurs via a pair of free radicals formed by adenosylcobalamin.[32] Electron paramagnetic resonance studies confirmed this mechanism by showing the radical intermediates that are formed from the hemolytic dissociation of the adenosylcobalamin cobalt-carbon bond.[33] *Propionibacterium shermanii* also showed radical intermediates by electron paramagnetic resonance, which is further proof of this mechanism.[34] Additionally UV-stopped flow spectroscopy shows that the hemolysis is coupled to hydrogen atom extraction from methylmalonyl-CoA, not adensylcobalamin.[35]

COFACTORS

Propionyl-CoA carboxylase requires biotin as a cofactor. Kogl first isolated biotin from egg white.[36] The structure of biotin was determined in 1942.[37] Biotin binds to the lysine of a well-defined binding site that has the sequence Ala-Met-Lys-Met.[24, 38] Propionyl-CoA carboxylase is biotinylated by holocarboxylase synthetase.[39]

Methylmalonyl-CoA mutase requires cobalamin as a cofactor[40, 41, 42]; more specifically the mutase requires a form of cobalamin called adenosylcobalamin. Cobalamin was first determined to be of interest in 1926 when Minot and Murphy[43] successfully treated pernicious anemia with a diet that included crude liver extracts. Two decades later, crude liver extract was again examined, and cobalamin isolated.[44] Rickes crystallized cobalamin from liver that same year, and also named it vitamin B12.[45] The structure of cobalamin was determined soon after by electron density mapping.[46] Cobalamin has many forms, but only the form adenosylcobalamin acts as a cofactor for methylmalonyl-CoA mutase.[25] The pathway from cobalamin to adenosylcobalamin was first described in *Clostridium tetanomorphum*, where it was also shown that magnesium was required.[47] It was later confirmed that a similar pathway occurs in mammals, as was shown by looking at the liver and kidneys of rabbit, rat, and human.[48] Kerwar also showed that cobalamin could be converted to adenosylcobalamin by HeLa cells, so long as they were cultured in the presence of ATP and in a reducing system.[49] This set of experiments also showed that the synthesis and cofactor activity was present in mitochondria.

GENE AND PROTEIN STRUCTURE

In 1986, the cDNA for propionyl-CoA carboxylase was first isolated.[50] Lamhonwah showed that the gene *PCCA*, responsible for propionyl-CoA carboxylase subunit α, was on chromosome 13; additionally, the gene *PCCB*, encoding propionyl-CoA carboxylase subunit β was on chromosome 3. This was further narrowed to *PCCA* being located at 13q32, and *PCCB* at 3q22.[51] They also noted that the α subunit mRNA was much larger than the β-subunit mRNA, with transcript lengths of 2.9 kb and 2.0 kb, respectively. The following year, a partial cDNA sequence was published, which showed the sequence for the biotin binding site and an approximately 60 amino acid conserved region that included the biotin binding site.[52] Other groups also published information gleaned about the individual subunits α[53, 54] and β[55, 56, 57, 58, 59], such as sequence fragments, amino acid length, exon boundaries, and pre- and post-processing size of the protein. The full cDNA sequence of each subunit was published soon after, which, for each, showed the complete structure of the 5'UTR, 3'UTR, and mitochondrial leader sequence.[60] Additional studies showed that the two subunits had unequal rates of synthesis or degradation, with the β-subunit being in excess.[61]

The methylmalonyl-CoA mutase gene was cloned in 1988 from human placenta.[62] The next year, this data was confirmed by cloning cDNA from human liver.[63] They showed that methylmalonyl-CoA mutase has a 742 amino acid open reading frame and a 32 amino acid leader sequence. Methylmalonyl-CoA mutase was shown to be located on chromosome 6, in the p12-21.1 region.[64, 65] The mutase is 13 exons long, and spans more than 35 kb of the genome. In 1986, Rosenberg also showed the mitochondrial targeting

sequence in detail, noting that it had a net positive charge, and that cleavage took place between a glutamine and an asparagine.[66] The trafficking of methylmalonyl-CoA mutase into the mitochondria is modulated by an energy-dependent mechanism; once in the mitochondria it is quickly converted into its mature form.[67] The presence or absence of adenosylcobalamin does not affect the localization of the mutase into the mitochondria.[68] The crystal structure of *Propionibacterium shermanii* shows that the C-terminal end is responsible for binding adenosylcobalamin, and the N-terminal end contains the homobinding domain.[69] The crystal structure of *Propionibacterium shermanii* has also helped elucidate how the cobalt atom is coordinated in the enzyme,[70] how methylmalonyl-CoA if found in bound and unbound forms[71], and what the structure looks like with and without a tyrosine at the active site[72].

CAUSE OF DISEASE STATES

Deficiencies resulting in disease

Cofactor deficiencies have been known to cause many different diseases. In the case of propionyl-CoA carboxylase, the cofactor is biotin. There is a report of experimental biotin deficiency[73] induced by biotin sequestration by consuming large amounts of egg white. The symptoms resulting from this diet were reversed with the application of biotin. Because methylmalonic acidemia has a cobalamin-responsive form (discussed below), it was postulated by Hsia that there might be a biotin responsive form of propionic acidemia.[74] However, there have been no reports of such a disease.

Methylmalonyl-CoA mutase requires the cofactor of adenosylcobalamin. There have been reports of cobalamin deficiency leading to methylmalonic acidemia.[ii, iii, 75] Most cases were readily reversed when cobalamin was administered, though some patients did not respond to cobalamin treatment. This result implicated methylmalonyl-CoA mutase as an additional location for metabolic defect, along with cobalamin deficiency.[95] It was also reported that homocystinuria occurs in patients with cobalamin-responsive methylmalonic acidemia.[76, 77] There has been one report of a child born with methylmalonic acidemia due to his vegetarian mother being cobalamin deficient during pregnancy.[78] The child was returned to health after cobalamin was added to his diet.

Genetic mutation resulting in disease

Retrospectively, Childs[i] was the first to identify propionic acidemia as a new disease. This disease state was further confirmed in 1968.[79] Studies used ^{14}C labeled propionate and showed that there was an error in propionate metabolism in patient fibroblasts[80] and leukocytes[li]. While propionyl-CoA carboxylase was clearly implicated as having a defect, this wasn't confirmed genetically until 1990[81] when the first of a slew of mutations were reported.[82, 83, 84, 85, 86] In the α-subunit, these mutations included 2 short insertion/deletions creating an exon skip, an insertion creating a premature stop codon, and mutations reducing the stability of the protein. In the β-subunit, mutations were found[81, 87, 88, 59] that cause the deletion of 14 basepairs with the insertion of 12 basepairs that creates a premature stop codon, an insertion leading to a premature stop codon, splicing mutations, and a mutation causing an exon skip. There is evidence for interallelic complementation with respect to the β-subunit.[89] The diversity in mutations that cause disease might be

responsible for the heterogeneity of symptoms and outcomes in propionic acidemia patients. Propionic acidemia is inherited in an autosomal recessive manner.[90]

There have been reports of patients who have a genetic defect in the enzyme that biotinylates propionyl-CoA carboxylase, holocarboxylase synthetase. Holocarboxylase synthetase also biotinylates Acetyl-CoA carboxylases 1 and 2, 3-methylcrotonyl-CoA carboxylase, pyruvate carboxylase.[91] Because holocarboxylase synthetase biotinylates these three additional enzymes, additional pathways are also affected. These patients have the disease multiple carboxylase deficiency.[92]

There has been one report of a patient with a methylmalonyl-CoA racemase deficiency.[93] This patient was restudied and confirmed to have a racemase mutation.[94]

In methylmalonic acidemia, methylmalonyl-CoA mutase was implicated by Smith and Monty in 1959.[40] This was further postulated in 1967[95] and 1968[96], when researchers came across patients with symptoms that were similar to cobalamin responsive patients, however, these new patients did not respond to cobalamin treatment. Genetically, the first mutations were found in 1990 when cDNA was cloned and sequenced from a patient.[97] Also that year, it was noted that the patients described to date did not have gross chromosomal rearrangements, large insertions or deletions, or mutations at restriction enzyme sites.[98] Since then many mutations have been found throughout the gene that cause either a mut^0 or mut^- phenotype.[99, 100, 101, 102, 103] Interestingly, there are some mut^0 cell lines that were able to complement some, but not all, mut^0 lines, giving evidence of interallelic complementation.[104] Because methylmalonyl-CoA mutase from *Propionibacterium shermanii* shares 65% sequence identity with human methylmalonyl-

CoA mutase, it can be used as a model to map mutations.[105] As such, Thoma showed that of the 12 known mutations at the time, eight were in the C-terminal adenosylcobalamin binding domain, and four were in the N-terminal homobinding domain.[69] More recently, a patient has been found with a mutation that results in a lack of targeting sequence for the protein.[106] Lastly, all of the mutations reported are inherited in an autosomal recessive manner, as evidenced by roughly equal numbers of males and females affected and lack of direct parental transmission of the disease.[95, 107] Many patients with methylmalonic acidemia are compound heterozygotes.[108]

Early on, it was reported that in addition to mutase deficiencies, abnormalities in cobalamin synthesis could lead to clinically significant methylmalonic acidemia..[109] Cox and White[ii] were the first to report on a patient whose symptoms were alleviated with cobalamin treatment. This was the first hint that cobalamin metabolism could lead to disease. Additional studies[110, 111] were done that showed that the primary defect in certain methylmalonic acidemia patients was in cobalamin metabolism. Early on it was known that there was more than one step that was affected, as 2 patients were shown to have different rates of accumulation of adenosylcobalamin, while retaining similar methylmalonyl-CoA to succinate turnover.[111] The first complementation tests showed that there were three complementation groups: *cblA*, *cblB*, and *cblC*.[112] Later studies confirmed the presence of a fourth group, *cblD*.[113] Further complementation tests showed that *cblA* and *cblC* are mutants for NADPH- and NADH-aquacobalamin reductases.[114] The *cblB* complementation group results from mutations in the cob(I)alamin adenosyltransferase.[115] Complementation tests have shown that there is some interallelic

complementation within the *cblA* group.[116] *cblA*, *cblB*, and *cblC* mutations are also inherited in an autosomal recessive manner.[107] More recently, *cblF* has been described.[117] However, due to low patient numbers in the *cblD* and *cblF* groups, the mode of inheritance has not been described definitively.

DISEASE MANIFESTATIONS

Propionic Acidemia

Propionic acidemia was first described as ketotic hyperglycinemia.[1] This patient[118, 119, 120, 121], and his sister[122] who was similarly affected, were studied extensively. The primary manifestations in these patients were vomiting, lethargy, ketosis, and hyperglycinemia. However, additional patients didn't consistently have the symptoms of ketosis[123], metabolic acidosis[124] or hyperglycinemia. In 1971, it was determined that ketotic hyperglycinemia and propionic acidemia were the same disease, and that the clinical presence of propionic acid, not ketosis or hyperglycinemia, was diagnostic for the disease.[125] A study of 65 cases revealed that the most common clinical symptoms were feeding difficulties, lethargy, and hypotonia, with less common symptoms of seizures, coma, and hepatomegaly, among others.[90] The hepatomegaly is due primarily to fat accumulation.[79] Long-term consequences include heart disease, metabolic stroke, pancreatitis, blindness, and hearing loss.

While propionic acidemia classically presents in the neonatal period, there are cases of "late onset" disease. Sutrees[126] compared those who presented early (within the

first week of life) with those who presented later (sixth week of life or later), and made the following observations. There was a higher death rate in the early onset group than the late onset group, each early onset patient had an IQ of less than 60 and many needed dialysis. In contrast, each of the late onset patients had an IQ of greater than 60, and movement disorders were common. Both groups had roughly equal proportion of patients with chorea and dystonia.

Patients with propionic acidemia have an characteristic biochemical profile including methylcitrate, propionylglycine, and 3-hydroxypropionate. Methylcitrate has been identified as a metabolite that was seen in patient, but not control populations.[127] Additionally, 3-hydroxypropionate has also been seen, which is likely a result of β-oxidation of propionate.[128] Tiglic aciduria has also been seen in patient samples.[129] Hyperammonemia is a common finding, and also correlates with propionic acid levels in serum.[130] It has been suggested that the hyperammonemia is due to propionic acid inhibiting ureagenesis when ammonia is the substrate.[131] More specifically, it has been suggested that the hyperammonemia is either due to depletion of N-acetyl glutamate, which, in turn, is attributable to depletion of acetyl-CoA or it is due competitive inhibition by propionyl–CoA of N-acetyl glutamate synthetase.[132] The latter hypothesis has been supported by additional studies.[133]

There are other interesting, but inconsistent, findings in patients with propionic acidemia. Neurologic symptoms are common.[134] There are reports of fatal symmetric necrosis of the basal ganglia.[124, 135] Additionally, there was a report of delayed neuronal

myelination upon inspection with an MRI.[136] One patient was also reported to have parathyroid hormone resistance and B-cell lymphopenia.[137]

Alternatively, there have are reports of propionyl-CoA carboxylase deficiencies in people who are asymptomatic. Wolf reported on a child whose propionyl-CoA carboxylase deficiency was not found until she was 13, when her brother was found to be symptomatic. While her urine did not have an abnormal organic acid profile, her fibroblasts were shown to have less than 10% enzyme activity.[138] This is contrasted with a boy who was discovered after an affected sibling was found. His cultured fibroblasts showed only 3% enzyme activity, and he was found to excrete methylcitrate, 3-hydroxypropionate, and glycine similar to his brother and other propionic acidemia patients.[139]

Methylmalonic Acidemia

Methylmalonic acidemia, the symptom, was first described in patients who had pernicious anemia, though pernicious anemia was a defect in cobalamin uptake and is distantly related to methylmalonic acidemia, the disease. Oberholzer showed that his patient excreted amounts of methylmalonic acid far in excess of pernicious anemia patients, and postulated that his patient had a new disease, which turned out to be methylmalonic acidemia due to a mutation in methylmalonyl-CoA mutase.[95] This was in contrast to cobalamin-responsive patients who had defects in cobalamin metabolism. However, cobalamin-responsive or not-responsive patients had similar symptoms. The two forms of the disease, cobalamin responsive and non-responsive, were described by Morrow in 1969.[140] He also stated that methylmalonic acid excretion was the hallmark of

this disease, and what differentiated it from ketotic hyperglycinemia (propionic acidemia). While methylmalonic acid excretion is a definitive diagnostic indicator for methylmalonic acidemia, studies show that excretion levels do not correlate with the clinical status of patients.[141]

Patients with methylmalonic acidemia present very similarly to patients with propionic acidemia. Symptoms include lethargy, failure to thrive, vomiting, dehydration, respiratory distress, hypotonia, and hepatomegaly.[107] More recently, patients with renal failure have been reported.[142, 143] As with propionic acidemia, neurologic symptoms have been reported in methylmalonic acidemia.[144, 145] Pancreatitis is also a chronic feature of this disease.[146] As noted earlier, there are two forms of methylmalonic acidemia due to methylmalonyl-CoA mutase defects, *mut*^{0} and *mut* $^{-}$. Patients with *mut*^{0} mutations have a more severe disease course, and a higher death rate than *mut* $^{-}$ patients.[147]

Methylmalonic acidemia patients who have cobalamin metabolism deficiencies have similar presentation to patients with methylmalonyl-CoA mutase deficiencies, but usually have a later onset, less mortality, and less severe course of disease.[147]

Biochemically, methylmalonic acidemia patients are also similar to propionic acidemia patients. Again, methylcitrate and 3-hydroxypropionate are seen,[127, 128] as well as propionic acid[148] and its derivatives.[149] Additionally, methylmalonic acid is an inhibitor of the malate-phosphate exchange, which may be responsible for the common findings of hyperglycemia and ketonemia.[150] Because ketotic hyperglycinemia is seen in both methylmalonic acidemia and propionic acidemia, it must be a secondary effect of CoA

metabolism.[151] Glutathione deficiency may contribute to lactic acidosis during decompensation episodes.[152]

As with propionic acidemia, there have been reports of children with defects in methylmalonyl-CoA mutase and high methylmalonic acid levels who are asymptomatic.[153, 154] All of these children were reported to have normal growth and development. Alternately, there was a report of a patient with "mild" methylmalonic acidemia, that is low-to-normal methylmalonic acid excretion, who had acidotic episodes and ketosis with hyperammonemia. Lastly, there have also been reports of children who have low-to-moderate methylmalonic acid excretion, as picked up by newborn screening, which usually resolved within two years of age.[155] Of those with persistent methylmalonic aciduria, but no metabolic episodes, all had normal somatic and cognitive outcomes.

DIAGNOSIS

Propionic acidemia and methylmalonic acidemia are usually diagnosed by measurement of their respective primary metabolite in urine or blood samples. However, prenatal testing has become available for both of these genetic disorders.

Propionic acidemia was first successfully diagnosed prenatally using cultured amniotic cells.[156] Propionyl-CoA carboxylase activity was not found in this culture, which was later confirmed using fibroblasts. Later, methylcitrate was used as a diagnostic marker in amniotic fluid.[157] Again, this was confirmed using propionyl-CoA carboxylase activity in cultured cells. Later, another prenatal diagnosis was made and

confirmed using chorionic villi.[158] Even more recently, acylcarnitines in amniotic fluid have been used as a diagnostic tool.[159]

Methylmalonic acidemia was similarly diagnosed successfully *in utero*. Morrow first detected methylmalonic acid in maternal urine and used that as evidence for a diagnosis.[160] This diagnosis was confirmed at birth. He later used ^{14}C incorporation in amniotic fluid cells to make a correct diagnosis.[161] Mahoney similarly used cultured amniotic fluid cells, but also showed that amniotic fluid methylmalonic acid concentration was diagnostic.[162]

TREATMENT

Prenatal treatment

With prenatal diagnosis comes the opportunity to start treatment. There have not been any reports of propionic acidemia being treated *in utero*. However, prenatal treatment of cobalamin responsive methylmalonic acidemia has been the subject of a few studies. In 1975, large doses of adenosylcobalamin were prescribed after culture of amniotic cells indicated a defect in cobalamin synthesis.[163] Additionally, another fetus, diagnosed in the third trimester responded well to large doses of adenosylcobalamin, as measured by maternal methylmalonic acid concentration.[164]

Patient treatment

Traditionally, the goals of propionic acidemia treatment were to lower propionic acid levels, and regulate ammonia, glycine, and alanine levels. Peritoneal dialysis has been

used to successfully ameliorate some biochemical symptoms, such as hyperammonemia, of propionic acidemia.[165] Diet is an important source of controlling propionate precursors. As such, parenteral nutrition can be useful to control intake when the patient is having a metabolic crises.[166] When patients are not under metabolic stress, maintenance treatment options include protein restriction, biotin supplementation[167] and L-carnitine supplementation.[168] L-carnitine supplementation has been shown to increase the formation and excretion of short chain acylcarnitines and patients who were treated improved clinically.[169] Another study of carnitine supplementation in conjunction with glycine showed an increase in tolerated protein, increased weight, development, and muscle tone, while showing a decrease in ketosis and hyperammonemia.[170] Another option is antibiotic treatment to reduce the amount of propionate from anaerobic gut bacteria.[171] In all patients, metronidazole was shown to reduce propionic acid excretion, and clinical improvement was seen in three of nine patients in this study.

Methylmalonic acidemia patients are treated similarly to propionic acid patients. Restriction of natural protein has been shown to require non-branch chain amino acid supplementation if the restriction is severe.[172] Cobalamin is administered to those patients who have cobalamin-responsive disease. With protein restriction and cobalamin doses, patients have done well.[173] L-carnitine has also been prescribed for methylmalonic acidemia patients.[169] In one patient who took supplemental carnitine, hippurate, which is a measure of mitochondrial ATP and CoA availability, increased along with a decrease in methylmalonyl and methylcitrate excretion.[174] Antibiotic treatment has also been used to reduce the propionate formed by natural flora, and subsequently, methylmalonic acid

excretion levels.[175] An additional antibiotic study showed decreases in hydroxypropionate, methylcitrate, and odd-chain fatty acid levels.[176]

Treatment of both acidemias has been largely supportive, and future studies should focus on finding improvements that impact patient health. Additionally, while the traditional method of restricting diet while administering carnitine and antibiotics has improved patient health[177], there is still a high probability of long-term complications.[178]

[1] Childs B, Nyhan WL, Borden M, Bard L, Cooke RE: Idiopathic hyperglycinemia and hyperglycinuria: New disorder of amino acid metabolism I. *Pediatrics* **27**:522,1961.

[2] Cox EV, White AM: Methylmalonic acid excretion: Index of vitamin-B12deficiency. *Lancet* **2**:853,1962.

[3] Lardy HA, Adler J: Synthesis of succinate from propionate and bicarbonate by soluble enzymes from liver mitochondria. *J Biol Chem* **219**:935,1956.

[4] Flavin M, Ortiz PJ, Ochoa S: Metabolism of propionic acid in animal tissues. *Nature* **176**:823,1955.

[5] Katz J, Chaikoff IL: The metabolism of propionate by rat liver slices and the formation of isosuccinic acid. *J Am Chem Soc* **77**:2659,1955.

[6] Beck WS, Flavin M, Ochoa S: Metabolism of propionic acid in animal tissues. III. Formation of Succinate. *J Biol Chem* **229**:997,1957.

[7] Flavin M, Ochoa S: Metabolism of propionic acid in animal tissues. I. Enzymatic conversion of propionate to succinate. *J Biol Chem* **229**:965,1957.

[8] Retey J, Lynen F: The absolute configuration of methylmalonyl-CoA. *Biochem Biophys Res Commun* **16**:358,1964.

[9] Sprecher M, Clark MJ, Sprinson DB: The absolute configuration of methylmalonyl-CoAand stereochemistry of the methylmalonyl-CoAmutase reaction. *Biochem Biophys Res Commun* **15**:581,1964.

[10] Mazumder R, Sasakawa T, Kaziro Y, Ochoa S: Metabolism of propionic acid in animal tissues. IX. Methylmalonyl coenzyme A racemase. *J Biol Chem* **237**:3065,1962.

[11] Thompson GN, Walter JH, Bresson J-L, Ford GC, Lyonnet SL, Chalmers RA, Saudubray J-M, Leonard JV, Halliday D: Sources of propionate in inborn errors of propionate metabolism. *Metabolism* **39**:1133,1990.

[12] Leonard JV: Stable isotope studies in propionic and methylmalonic acidaemia. Eur J Pediatr 156 (suppl):67, 1997.

[13] Tanaka K, Armitage IM, Ramsdell HS, Hsia YE, Lipsky SR, Rosenberg LE: [13C]Valine metabolism in methylmalonicacidemia using nuclear magnetic resonance: Propionate as an obligate intermediate. *Proc Natl Acad Sci U S A* **72**:3692,1975.

[14] Baretz BH, Tanaka K: Metabolism in rats *in vivo* of isobutyrates labelled with stable isotopes at various positions: Identification of propionate as an obligate Intermediate. *J Biol Chem* **253**:4203,1978.

[15] Tietz A, Ochoa S: Metabolism of propionic acid in animal tissues. V. Purification and properties of propionyl Carboxylase. *J Biol Chem* **234**:1394,1959.

[16] Kaziro Y, Ochoa S, Warner RC, Chen J: Metabolism of propionic acid in animal tissues. VIII. Crystalline propionyl carboxylase. *J Biol Chem* **236**:1917,1961.

[17] Lau EP, Cochran BC, Munson L, Fall RR: Bovine kidney 3-methylcrotonyl-CoAand propionyl-CoAcarboxylases: Each enzyme contains nonidentical subunits. *Proc Natl Acad Sci U S A* **76**:214,1979.

[18] Kalousek F, Darigo MD, Rosenberg LE: Isolation and characterization of propionyl-CoAcarboxylase from normal human liver: Evidence for a protomeric tetramer of nonidentical subunits. *J Biol Chem* **255**:60,1980.

[19] Gravel RA, Lam KF, Mahuran D, Kronis A: Purification of human liver propionyl-CoAcarboxylase by carbon tetrachloride extraction and monomeric avidin affinity chromatography. *Arch Biochem Biophys* **201**:669,1980

[20] Hsia YE, Scully KJ, Rosenberg LE: Human propionylCoAcarboxylase: Some properties of the partially purified enzyme in fibroblasts from controls and patients with propionic acidemia. *Pediatr Res* **13**:746,1979.

[21] Haase FC, Beegen H, Allen SHK: Propionyl-coenzyme A carboxylase of *Mycobacterium smegmatis* : An electron microscopic study. *Eur J Biochem* **140**:147,1984.

[22] Wolf B, Hsia YE, Rosenberg LE: Biochemical differences between mutant propionyl-CoAcarboxylases from two complementation groups. *Am J Hum Genet* **30**:455,1978.

[23] Mistry SP, Dakshinamurti K: Biochemistry of biotin. *Vitam Horm* **22**:1,1964.

[24] Lamhonwah AM, Quan F, Gravel RA: Sequence homology around the biotin-binding site of human propionyl-CoAcarboxylase and pyruvate carboxylase. *Arch Biochem Biophys* **254**:631,1987.

[25] Weissbach H, Toohey J, Barker HA: Isolation and properties of B12coenzymes containing benzimidazole or dimethylbenzimidazole. *Proc Natl Acad Sci U S A* **45**:521,1959.

[26] Rosenberg LE, Lilljeqvist AC, Hsia YE, Rosenbloom FM: Vitamin B12dependent methylmalonicaciduria: defective B12metabolism in cultured fibroblasts. *Biochem Biophys Res Commun* **37**:607,1969.

[27] Kolhouse JF, Utley C, Allen RH: Isolation and characterization of methylmalonyl-CoAmutase from human placenta. *J Biol Chem* **255**:2708,1980.

[28] Fenton WA, Hack AM, Willard HF, Gertler A, Rosenberg LE: Purification and properties of methylmalonylCoAmutase from human liver. *Arch Biochem Biophys* **214**:815,1982.

[29] Willard HF, Rosenberg LE: Interactions of methylmalonylCoAmutase from normal human fibroblasts with adenosylcobalamin and methylmalonylCoA: Evidence for nonequivalent active sites. *Arch Biochem Biophys* **200**:130,1980.

[30] Kellermeyer RW, Wood HG: Methylmalonyl isomerase: A study of the mechanism of isomerization. *Biochemistry* **1**:1124,1962.

[31] Phares EF, Long MV, Carson SF: An intramolecular rearrangement in the methylmalonyl isomerase reaction as demonstrated by positive and negative mass analysis of succinic acid. *Biochem Biophys Res Commun* **8**:142,1962.

[32] Halpern J: Mechanisms of coenzyme B12-dependent rearrangements. *Science* **227**:869,1985.

[33] Zhao Y, Abend A, Kunz M, Such P, Retey J: Electron paramagnetic resonance studies of the methylmalonyl-CoAmutase reaction. *Eur J Biochem* **225**:891,1994.

[34] Padmakumar R, Banerjee R: Evidence from electron paramagnetic resonance spectroscopy of the participation of radical intermediates in the reaction catalyzed by methylmalonyl-coenzyme A mutase. *J Biol Chem* **270**:9295,1995.

[35] Padmakumar R, Banerjee R: Evidence that cobalt–carbon bond homolysis is coupled to hydrogen atom abstration from substrate in methylmalonyl-CoAmutase. *Biochemistry* **36**:3713,1997.

[36] Kogl F, Tonis B: Uber das Bios-Problem. Darstellung und Krystallisiertem biotin aus Eigelb. *Z Physiol Chem* **242**:43,1936.

[37] du Vigneaud V, Hoffmann K, Melville DB: On the structure of biotin. *J Am Chem Soc* **64**:188,1942.

[38] Browner MF, Taroni F, Sztul E, Rosenberg LE: Sequence analysis, biogenesis, and mitochondrial import of the α-subunit of rat liver propionyl-CoAcarboxylase. *J Biol Chem* **264**:12680,1989.

[39] Chiba Y, Suzuki Y, Aoki Y, Ishida Y, Narisawa K: Purification and properties of bovine liver holocarboxylase synthetase. *Arch Biochem Biophys* **313**:8,1994.

[40] Smith RM, Monty KJ: Vitamin B12and propionate metabolism. *Biochem Biophys Res Commun* **1**:105,1959.

[41] Gurnani S, Mistry SP, Johnson BC: Function of vitamin B12in methylmalonate metabolism. 1. Effect of a cofactor form of B12on the activity of methylmalonyl-CoAisomerase. *Biochim Biophys Acta* **38**:187,1960.

[42] Stern JR, Friedmann DC: Vitamin B12and methylmalonyl-CoAisomerase. I. Vitamin B12and propionate metabolism. *Biochem Biophys Res Commun* **2**:82,1960.

[43] Minot GR, Murphy LP: Treatment of pernicious anemia by a special diet. *JAMA* **87**:470,1926.

[44] Smith EL: Purification of anti-pernicious anemia factors from liver. *Nature* **161**:638,1948.

[45] Rickes EL, Brink NG, Koniuszy FR, Wood TR, Folkers K: Crystalline vitamin B12. *Science* **107**:396,1948.

[46] Hodgkin DC, Kamper J, Mackay M, Pickworth J, Trueblood KN, White JG: Structure of vitamin B12. *Nature* **178**:64,1956.

[47] Vitols E, Walker GA, Huennekens FM: Enzymatic conversion of vitamin B12to a cobamide coenzyme, a (5,6-dimethylbenzimidazolyl) deoxyadenosylcobamide (adenosyl-B12). *J Biol Chem* **241**:1455,1966.

[48] Pawalkiewicz J, Gorna M, Fenrych W, Magas S: Conversion of cyanocobalamin *in vivo* and *in vitro* into its coenzyme form in humans and animals. *Ann N Y Acad Sci* **112**:641,1964.

[49] Kerwar SS, Spears C, McAuslan B, Weissbach H: Studies on vitamin B12metabolism in HeLa cells. *Arch Biochem Biophys* **142**:231,1971.

[50] Lamhonwah AM, Barankiewics TJ, Willard HF, Mahuran DJ, Quan F, Gravel RA: Isolation of cDNA clones coding for the α and β chains of human propionyl-

CoAcarboxylase: Chromosomal assignments and DNA polymorphisms associated with PCCA and PCCB genes. *Proc Natl Acad Sci U S A* **83**:4864,1986

[51] Leon-del-Rio A, Gravel RA: Sequence requirements for the biotinylation of carboxyl-terminal fragments of human propionyl-CoAcarboxylase alpha subunit expressed in *Escherichia coli*. *J Biol Chem* **269**:22964,1994.

[52] Lamhonwah AM, Quan F, Gravel RA: Sequence homology around the biotin-binding site of human propionyl-CoAcarboxylase and pyruvate carboxylase. *Arch Biochem Biophys* **254**:631,1987.

[53] Browner MF, Taroni F, Sztul E, Rosenberg LE: Sequence analysis, biogenesis, and mitochondrial import of the α-subunit of rat liver propionyl-CoAcarboxylase. *J Biol Chem* **264**:12680,1989.

[54] Leon-del-Rio A, Gravel RA: Sequence requirements for the biotinylation of carboxyl-terminal fragments of human propionyl-CoAcarboxylase alpha subunit expressed in *Escherichia coli*. *J Biol Chem* **269**:22964,1994.

[55] Kraus JP, Williamson CL, Firgaira FA, Yang-Feng TL, Munke M, Francke U, Rosenberg LE: Cloning and screening with nanogram amounts of immunopurified messenger RNAs: cDNA cloning and chromosomal mapping of cystathionine β-synthase and the β-subunit of propionylCoAcarboxylase. *Proc Natl Acad Sci U S A* **83**:2047,1986.

[56] Kraus JP, Firgaira F, Novotny J, Kalousek F, Williams KR, Williamson C, Ohura T , et al.: Coding sequence of the precursor of the β subunit of rat propionyl-CoAcarboxylase. *Proc Natl Acad Sci U S A* **83**:8049,1986.

[57] Kraus JP, Kalousek F, Rosenberg LE: Biosynthesis and mitochondrial processing of the β subunit of propionylCoAcarboxylase from rat liver. *J Biol Chem* **258**:7245,1983.

[58] Rodriguez-Pombo P, Hoenicka J, Muro S, Perez B, Perezcerda C, Richard E, Desviat LR , et al.: Human propionyl-CoAcarboxylase beta subunit gene—Exon-intron definition and mutation spectrum in Spanish and Latin American propionic acidemia. *Am J Hum Genet* **63**:360,1998.

[59] Ohura T, Ogasawara M, Ikeda H, Narisawa K, Tada K: The molecular defect in propionic acidemia: Exon skipping caused by an 8-bp deletion from an intron in the PCCB allele. *Hum Genet* **92**:397,1993.

[60] Lamhonwah AM, Mahuran D, Gravel RA: Human mitochondrial propionyl-CoAcarboxylase: Localization of the N-terminus of the pro- and mature alpha chains in the deduced primary sequence of a full-length cDNA. *Nucleic Acids Res* **17**:4396,1989

[61] Wolf B, Rosenberg LE: Heterozygote expression in propionyl coenzyme A carboxylase deficiency: Differences between major complementation groups. *Clin Invest* **62**:931,1978.

[62] Ledley FD, Lumetta M, Nguyen PN, Kolhouse JF, Allen RH: Molecular cloning of L-methylmalonyl-CoAmutase: Gene transfer and analysis of cell lines. *Proc Natl Acad Sci U S A* **85**:3518,1988.

[63] Jansen R, Kalousek F, Fenton WA, Rosenberg LE, Ledley FD: Cloning of full-length methylmalonyl-CoAmutase from a cDNA library using the polymerase chain reaction. *Genomics* **4**:198,1989.

[64] Nham S-U, Wilkemeyer MF, Ledley FD: Structure of the human methylmalonyl-CoA mutase (*MUT*) locus. *Genomics* **8**:710,1990.

[65] Ledley FD, Lumetta MR, Zoghli HY, Van Tuinen P, Ledbetter SA, Ledbetter DH: Mapping of human methylmalonylCoA mutase (MUT) locus on chromosome 6. *Am J Hum Genet* **42**:839,1988.

[66] Rosenberg LE, Fenton WA, Horwich AL, Kalousek F, Kraus JP: Targeting of nuclear-encoded proteins to the mitochondrial matrix: Implications for human genetic defects. *Ann N Y Acad Sci* **488**:99,1986.

[67] Fenton WA, Hack AM, Helfgott D, Rosenberg LE: Biogenesis of the mitochondrial enzyme methylmalonyl-CoAmutase: Synthesis and processing of a precursor in a cell-free system and in cultured cells. *J Biol Chem* **259**:6616,1984.

[68] Frenkel EP, Kitchens RL: Intracellular localization of hepatic propionyl-CoAcarboxylase and methylmalonyl-CoAmutase in humans and normal and vitamin B12deficient rats. *Br J Haematol* **31**:501,1975.

[69] Thoma NH, Leadley PF: Homology modelling of human methylmalonyl-CoAmutase: A structural basis for point mutations causing methylmalonic aciduria. *Protein Sci* **5**:1922,1996.

[70] Mancia F, Keep NH, Nakagawa A, Leadley PF, McSweeney S, Rasmussen B, Bosecke P, Diat O, Evans PR: How coenzyme B12radicals are generated: The crystal structure of methylmalonyl-coenzyme A mutase at 2 A resolution. *Structure* **4**:339,1996.

[71] Mancia F, Evans PR: Conformational changes on substrate binding to methylmalonylCoAmutase and new insights into the free radical mechanism. *Structure* **6**:711,1998.

[72] Thoma NH, Meier TW, Evans PR, Leadlay PF: Stabilization of radical intermediates by an active-site tyrosine residue in methylmalonyl-CoAmutase. *Biochemistry* **37**:14386,1998.

[73] Sydenstricker VP, Singal SA, Briggs AP, DeVaughn NM: Preliminary observations on "egg white injury" in man and its cure with a biotin concentrate. *Science* **95**:1976,1942.

[74] Hsia YE, Scully KJ, Rosenberg LE: Defective propionate carboxylation in ketotic hyperglycinaemia. *Lancet* **1**:757,1969.

[75] Barness LA, Young D, Mellman WJ, Kahn SB, Williams WJ: Methylmalonate excretion in patients with pernicious anemia. *New Engl J Med* **268**:144,1963.

[76] Shipman RT, Townley RRW, Danks DM: Homocystinuria, addisonian pernicious anaemia, and partial deletion of a G chromosome. *Lancet* **2**:693,1969.

[77] Hollowell JG, Jr, Hall WK, Coryell ME, McPherson JJ, Hahn DA: Homocystinuria and organic aciduria in a patient with vitamin-B12deficiency. *Lancet* **2**:1428,1969.

[78] Higginbottom MC, Sweetman L, Nyhan WL: A syndrome of methylmalonic aciduria, homocystinuria, megaloblastic anemia and neurologic abnormalities in a vitamin B12-deficient breast-fed infant of a strict vegetarian. *New Engl J Med* **299**:317,1978.

[79] Hommes FA, Kuipers JRG, Elema JD, Janse JF, Jonxis JJP: Propionicacidemia, a new inborn error of metabolism. *Pediatr Res* **2**:519,1968.

[80] Hsia YE, Scully KJ, Rosenberg LE: Inherited propionyl-CoAcarboxylase deficiency in "ketotic hyperglycinemia." *J Clin Invest* **50**:127,1971.

[81] Tahara T, Kraus JP, Rosenberg LE: An unusual insertion/deletion in the gene encoding the β-subunit of propionyl-CoAcarboxylase is a frequent mutation in caucasian propionic acidemia. *Proc Natl Acad Sci U S A* **87**:1372,1990.

[82] Tahara T, Kraus JP, Ohura T, Rosenberg LE, Fenton WA: Three independent mutations in the same exon of the PCCB gene: Differences between caucasian and Japanese propionic acidaemia. *J Inherit Metab Dis* **16**:353,1993.

[83] Richard E, Desviat LR, Perez-Cerda C, Ugarte M: Three novel splice mutations in the PCCA gene causing identical exon skipping in propionic acidemia patients. *Hum Genet* **101**:93,1997.

[84] Campeau E, Dupuis L, Leclere D, Gravel RA: Detection of a normally rare transcript in propionic acidemia patients with mRNA destabilizing mutations in the PCCA gene. *Hum Mol Genet* **8**:107,1999.

[85] Richard E, Desviat LR, Perez B, Perez-Cerda C, Ugarte M: Genetic heterogeneity in propionic acidemia patients with alpha-subunit defects. Identification of five novel mutations, one of them causing instability of the protein. *Biochim Biophys Acta* **1453**:351,1999.

[86] Campeau E, Dupuis L, Leon-del-Rio A, Gravel R: Coding sequence mutations in the alpha subunit of propionyl-CoAcarboxylase in patients with propionic acidemia. *Mol Genet Metab* **67**:1,1999.

[87] Ohura T, Narisawa K, Tada K: Propionic acidaemia: Sequence analysis of mutant mRNAs from Japanese beta subunit-deficient patients. *J Inherit Metab Dis* **16**:863,1993.

[88] Ohura T, Narisawa K, Tada K, Iinuma K: A novel splicing mutation in propionic acidemia associated with a tetranucleotide direct repeat in the PCCB gene. *Hum Genet* **95**:707,1995.

[89] Gravel RA, Akerman BR, Lamhonwah A-M, Loyer M, Leon-del-Rio A, Italiano I: Mutations participating in interallelic complementation in propionic acidemia. *Am J Hum Genet* **55**:51,1994.

[90] Wolf B, Hsia YE, Sweetman L, Gravel R, Harris DJ, Nyhan WL: Propionic acidemia: A clinical update. *J Pediatr* **99**:835,1981.

[91] Esaki S, Malkaram SA, ZempleniJ: Effects of single-nucleotide polymorphisms in the human holocarboxylase synthetase gene on enzyme catalysis. *Eur J Hum Gen* **20**: 428, 2012

[92] Baumgartner ER, Suormala T: Multiple carboxylase deficiency: Inherited and acquired disorders of biotin metabolism. *Int J Vitam Nutr Res* **67**:377,1997.

[93] Kang ES, Snodgrass PJ, Gerald PS: Methylmalonyl coenzyme A racemase defect: Another cause of methylmalonic aciduria. *Pediatr Res* **6**:875,1972.

[94] Willard HF, Rosenberg LE: Inherited deficiencies of human methylmalonyl CoA mutase: Biochemical and genetic studies in cultured skin fibroblasts, in Hommes FA (ed): Models for the Study of Inborn Errors of Metabolism. Amsterdam, Elsevier North-Holland, 1979, p 297.

[95] Oberholzer VC, Levin B, Burgess EA, Young WF: Methylmalonic aciduria: An inborn error of metabolism leading to chronic metabolic acidosis. *Arch Dis Child* **42**:492,1967.

[96] Rosenberg LE, Lilljeqvist A-C, Hsia YE: Methylmalonic aciduria: An inborn error leading to metabolic acidosis, long-chain ketonuria and intermittent hyperglycinemia. *New Engl J Med* **278**:1319,1968.

[97] Sethi KD, Ray R, Roesel RA, Carter AL, Gallagher BB, Loring DW, Hommes FA: Adult-onset chorea and dementia with propionic acidemia. *Neurology* **39**:1343,1989.

[98] Ledley FD, Crane AM, Lumetta M: Heterogeneous alleles and expression of methylmalonylCoAmutase inmethylmalonic acidemia. *Am J Hum Genet* **6**:539,1990.

[99] Adjalla CE, Hosack AR, Gilfix BM, Sun S, Chan A, Evans S, Matiaszuk NV , et al.: Seven novel mutations in mut methylmalonic aciduria. *Hum Mut* **11**:270,1998.

[100] Adjalla CE, Hosack AR, Matiaszuk NV, Rosenblatt DS: A common mutation among blacks with mut−methylmalonic aciduria. *Hum Mut Suppl* **1**:248,1998.

[101] Crane A, Martin LS, Valle D, Ledley FD: Phenotype of disease in three patients with identical mutations in methylmalonylCoAmutase. *Hum Genet* **89**:259,1992.

[102] Ogasawara M, Matsubara Y, Mikami H, Narisawa KK: Identification of two novel mutations in the methylmalonyl-CoAmutase gene with decreased levels of mutant mRNA in methylmalonic acidemia. *Hum Mol Genet* **3**:867,1994.

[103] Janata J, Kogekar N, Fenton WA: Expression and kinetic characterization of methylmalonyl-CoAmutase from patients with the−phenotype: Evidence for naturally occurring interallelic complementation. *Hum Mol Genet* **6**:1457,1997.

[104] Raff ML, Crane AM, Jansen R, Ledley FD, Rosenblatt DS: Genetic characterization of a MUT locus mutatation discriminating heterogeneity inmutoand–methymalonic aciduria by interallelic complementation. *J Clin Invest* **87**:203,1991.

[105] Thoma NH, Leadley PF: Homology modelling of human methylmalonyl-CoAmutase: A structural basis for point mutations causing methylmalonic aciduria. *Protein Sci* **5**:1922,1996.

[106] Ledley FD, Jansen R, Nham SU, Fenton WA, Rosenberg LE: Mutation eliminating mitochondrial leader sequence of methylmalonyl-CoAmutase causes0methylmalonic aciduria. *Proc Natl Acad Sci U S A* **87**:3147,1990.

[107] Matsui SM, Mahoney MJ, Rosenberg LE: The natural history of the inherited methylmalonic acidemias. *New Engl J Med* **308**:857,1983.

[108] Willard HF, Rosenberg LE: Inherited methylmalonylCoAmutase apoenzyme deficiency in human fibroblasts: Evidence for allelic heterogeneity, genetic compounds, and codominant expression. *J Clin Invest* **65**:690,1980.

[109] Willard HF, Rosenberg LE: Inherited deficiencies of human methylmalonyl CoA mutase: Biochemical and genetic studies in cultured skin fibroblasts, in Hommes FA (ed): Models for the Study of Inborn Errors of Metabolism. Amsterdam, Elsevier North-Holland, 1979, p 297.

[110] Rosenberg LE, Lilljeqvist AC, Hsia YE, Rosenbloom FM: Vitamin B12dependent methylmalonicaciduria: defective B12metabolism in cultured fibroblasts. *Biochem Biophys Res Commun* **37**:607,1969.

[111] Mahoney MJ, Rosenberg LE, Mudd SH, Uhlendorf BW: Defective metabolism of vitamin B12in fibroblasts from children with methylmalonicaciduria. *Biochem Biophys Res Commun* **44**:375,1971.

[112] Gravel RA, Mahoney MJ, Ruddle FH, Rosenberg LE: Genetic complementation in heterokaryons of human fibroblasts defective in cobalamin metabolism. *Proc Natl Acad Sci U S A* **72**:3181,1975.

[113] Willard HF, Mellman IS, Rosenberg LE: Genetic complementation among inherited deficiencies of methylmalonyl-CoAmutase activity: Evidence for a new class of human cobalamin mutant. *Am J Hum Genet* **30**:1,1978.

[114] Watanabe F, Saido H, Yamaji R, Miyatake K, Isegawa Y, Ito A, Yubisui T , et al.: Mitochondrial NADH- or NADP-linked aquacobalamin reductase activity is low in human skin fibroblasts with defects in synthesis of cobalamin coenzymes. *J Nutr* **126**:2947,1996.

[115] Fenton WA, Rosenberg LE: The defect in the cbl B class of human methylmalonic acidemia: Deficiency of cob(I)alamin adenosyltransferase activity in extracts of cultured fibroblasts. *Biochem Biophys Res Commun* **98**:283,1981.

[116] Cooper BA, Rosenblatt DS, Watkins D: Methylmalonic aciduria due to a new defect in adenosylcobalamin accumulation by cells. *Am J Hematol* **34**:115,1990.

[117] Watkins D, Rosenblatt DS: Failure of lysosomal release of vitamin B12: A new complementation group causing methylmalonic aciduria (cblF). *Am J Hum Genet* **39**:404,1986.

[118] Nyhan WL, Borden M, Childs B: Idiopathic hyperglycinemia: A new disorder of amino acid metabolism. II. The concentrations of other amino acids in the plasma and their modification by the administration of leucine. *Pediatrics* **27**:539,1961.

[119] Childs B, Nyhan WL, Brandt IK, Hsia YE, Clement DH, Provence SA: Further observations of a patient with hyperglycinemia Propionicacidemia (ketotic hyperglycinemia): Dietary treatment results in normal growth and development. *Pediatrics* **53**:391,1974.

[120] Nyhan WL, Childs B: Hyperglycinemia. V. The miscible pool and turnover rate of glycine and the formation of serine. *J Clin Invest* **43**:2404,1964.

[121] Menkes JH, Idiopathic hyperglycinemia: Isolation and identification of three previously undescribed urinary ketones : *J Pediatr* **69**:413,1966.

[122] Brandt IK, Hsia YE, Clement DH, Provence SA: Propionic acidemia (ketotic hyperglycinemia): Dietary treatment results in normal growth and development. *Pediatrics* **53**:391,1974.

[123] Mahoney MJ, Hsia YE, Rosenberg LE: Propionyl-CoAcarboxylase deficiency (propionicacidemia): A cause of non-ketotic hyperglycinemia. *Pediatr Res* **5**:395,1971.

[124] Perez-Cerda C, Merinero B, Marti M, Cabrera JC, Pena L, Garcia MJ, Gangoiti J , et al.: An unusual late-onset case of propionic acidaemia: Biochemical investigations, neuroradiological findings and mutation analysis. *Eur J Pediatr* **157**:50,1998.

[125] Ando T, Rasmussen K, Nyhan WL, Donnell GN, Barnes ND: Propionicacidemia in patients with ketotic hyperglycinemia. *J Pediatr* **78**:827,1971.

[126] Surtees RAH, Matthews EE, Leonard JV: Neurologic outcome of propionic acidemia. *Pediatr Neurol* **8**:333,1992.

[127] Ando T, Rasmussen K, Wright JM, Nyhan WL: Isolation and identification of methylcitrate, a major metabolic product of propionate in patients with propionic acidemia. *J Biol Chem* **247**:2200,1972.

[128] Ando T, Rasmussen K, Nyhan WL, Hull D: 3-Hydroxypropionate: Significance of β-oxidation of propionate in patients with propionic acidemia and methylmalonic acidemia. *Proc Natl Acad Sci U S A* **69**:2807,1972

[129] Nyhan WL, Ando T, Rasmussen K, Wadlington W, Kilroy AW, Cottom D, Hull D: Tiglic aciduria in propionic-acidemia. *Biochem J* **126**:1035,1972.

[130] Wolf B, Hsia YE, Tanaka K, Rosenberg LE: Correlation between serum propionate and blood ammonia concentrations in propionic acidemia. *J Pediatr* **93**:471,1978.

[131] Glasgow AM, Chase HP: Effect of propionic acid on fatty acid oxidation and ureagenesis. *Pediatr Res* **10**:683,1976.

[132] Stewart PM, Walser M: Failure of the normal ureagenic response to amino acids in organic acid loaded rats: A proposed mechanism for the hyperammonemia of propionic and methylmalonic acidemia. *J Clin Invest* **66**:484,1980.

[133] Coude FX, Sweetman L, Nyhan WL: Inhibition by propionylCoAof *N* -acetylglutamate synthetase in rat liver mitochondria. *J Clin Invest* **64**:1544,1979.

[134] Walter JH, Wraith JE, Cleary MA: Absence of acidosis in the initial presentation of propionic acidaemia. *Arch Dis Child (Fetal and Neonatal Edition)* **72**:F197,1995

[135] Haas RH, Marsden DL, Capistrano-Estrada S, Hamilton R, Grafe MR, Wong W, Nyhan WL: Acute Basal Ganglia Infarction in Propionic Acidemia. *J Child Neurol* **10**:18, 1995

[136] Bergman AJIW, Van Der Knaap MS, Smeitink JAM, Duran M, Dorland L, Valk J, Poll-The BT: Magnetic resonance imaging and spectroscopy of the brain in propionic acidemia: Clinical and biochemical considerations. *Pediatr Res* **40**:404,1996.

[137] Griffin TA, Hostoffer RW, Tserng KY, Lebovitz DJ, Hoppel CL, Mosser JL, Kaplan D , et al.: Parathyroid hormone resistance and B cell lymphopenia in propionic acidemia. *Acta Paediatr* **85**:875,1996.

[138] Wolf B, Paulsen EP, Hsia YE: Asymptomatic propionylCoAcarboxylase deficiency in a 13-year-old girl. *J Pediatr* **95**:563,1979.

[139] Inoue Y, Matsumoto I: Urinary acid profiles of asymptomatic propionylCoAcarboxylase deficiency. *J Pediatr* **113**:787,1988.

[140] Morrow G, Barness LA, Auerbach VH, Di George AM, Ando T, Nyhan WL: Observations on the coexistence of methylmalonic acidemia and glycinemia. *J Pediatr* **74**:680,1969.

[141] Thompson GN, Walter JH, Bresson JL, Ford GC, Bonnefont JP, Chalmers RA, Saudubray JM et al.: Substrate disposal in metabolic disease: A comparison between rates of *in vivo* propionate oxidation and urinary metabolite excretion in children with methylmalonic acidemia. *J Pediatr* **115**:735,1989.

[142] Rutledge SL, Geraghty M, Mroczek E, Rosenblatt D, Kohout E: Tubulointerstitial nephritis in methylmalonic aciduria. *Pediatr Nephrol* **7**(1):81,1993.

[143] Walter JH, Michalski A, Wilson WM, Leonard JV, Barratt TM, Dillon MJ: Chronic renal failure in methylmalonic acidaemia. *Eur J Pediatr* **148**:344,1989.

[144] Korf B, Wallman JK, Levy HL: Bilateral lucency of the globus pallidus complicating methylmalonic acidemia. *Ann Neurol* **20**:364,1986.

[145] Thompson GN, Christodoulou J, Danks DM: Metabolic stroke in methylmalonic acidemia. *J Pediatr* **115**:499,1989.

[146] Marquard J, El Scheich T, Klee D, Schmitt M, Meissner T, Mayatepek E, Oh J: Chronic pancreatitis in branched-chain organic acidurias--a case of methylmalonic aciduria and an overview of the literature. *Eur J Pediatr* **170**: 241, 2011.

[147] Nicolaides P, Leonard J, Surtees R: Neurological outcome of methylmalonic acidaemia. *Arch Dis Child* **78**:508,1998.

[148] Cox EV, Robertson-Smith D, Small M, White AM: The excretion of propionate and acetate in vitamin B12deficiency. *Clin Sci* **35**:123,1968.

[149] Wendel U, Eissler A, Sperl W, Schadewaldt P: On the differences between urinary metabolite excretion and odd-numbered fatty acid production in propionic and methylmalonic acidaemias. *J Inher Metab Dis* **18**:584,1995.

[150] Halperin ML, Schiller CM, Fritz IB: The inhibition by methylmalonic acid of malate transport by the dicarboxylate carrier in rat liver mitochondria: A possible explanation for hypoglycemia in methylmalonic aciduria. *J Clin Invest* **50**:2276,1971.

[151] Gompertz D, Storrs CN, Bau DCK, Peters TJ, Hughes EA: Localization of enzyme defect in propionicacidemia. *Lancet* **1**:1140,1970.

[152] Treacy E, Arbour L, Chessex P, Graham G, Kasprzak L, Casey K, Bell L , et al.: Glutathione deficiency as a complication of methylmalonic acidemia, response to high ascorbate. *J Pediatr* **129**:445,1996.

[153] Ledley FD, Levy HL, Shih VE, Benjamin R, Mahoney MJ: Benign methylmalonic aciduria. *New Engl J Med* **311**:1015,1984.

[154] Coulombe JT, Shih VE, Levy HL: Massachusetts Metabolic Disorders Screening Program. II. Methylmalonic aciduria. *Pediatrics* **67**:26,1981.

[155] Sniderman LC, Lambert M, Giguere R, Auray-Blais C, Lemieux B, Laframboise R, Rosenblatt DS , et al.: Outcome of individuals with low-moderate methylmalonic aciduria detected through a neonatal screening program. *J Pediatr* **134**:680,1999.

[156] Ledley FD, Rosenblatt DS: Mutations in mut methylmalonic acidemia: Clinical and enzymatic correlations. *Hum Mut* **9**:1,1997.

[157] Sweetman L, Weyler W, Shafai T, Young PE, Nyhan WL: Prenatal diagnosis of propionic acidemia. *JAMA* **242**:1048,1979.

[158] Chadefaux B, Augereau C, Rabier D, Rocchiccioli F, Boué J, Oury JF, Kamoun P: Prenatal diagnosis of propionic acidaemia in chorionic villi by direct assay of propionyl-CoAcarboxylase. *Prenat Diagn* **8**:161,1988.

[159] Van Hove JLK, Chace DH, Kahler SG, Millington DS: Acylcarnitines in amniotic fluid: Application to the prenatal diagnosis of propionic acidaemia *J Inherit Metab Dis* **16**:361, 1993

[160] Morrow G, Schwartz RH, Hallock JA, Barness LA: Prenatal detection of methylmalonic acidemia. *J Pediatr* **77**:120,1970.

[161] Morrow G, Revsin B, Mathews C, Giles H: A simple rapid method for prenatal detection of defects in propionate metabolism. *Clin Genet* **10**:218,1976.

[162] Mahoney MJ, Rosenberg LE, Linblad B, Waldenstrom J, Zetterstrom R: Prenatal diagnosis of methylmalonic aciduria. *Acta Paediatr Scand* **64**:44,1975.

[163] Ampola MG, Mahoney MJ, Nakamura E, Tanaka K: Prenatal therapy of a patient with vitamin B12responsive methylmalonic acidemia. *New Engl J Med* **293**:313,1975.

[164] Van der Meer SB, Spaapen LJM, Fowler B, Jakobs C, Kleijer WJ, Wendel U: Prenatal treatment of a patient with vitamin B12-responsive methylmalonic acidemia. *J Pediatr* **117**:923,1990.

[165] Gortner L, Leupold D, Pohlandt F, Bartmann P: Peritoneal dialysis in the treatment of metabolic crises caused by inherited disorders of organic and amino acid metabolism. *Acta Paediatr Scand* **78**:706,1989.

[166] Kahler SG, Millington DS, Cederbaum SD, Vargas J, Bond LD, Maltby DA, Gale DS, Roe CR: Parenteral nutrition in propionic and methylmalonic acidemia. *J Pediatr* **15**:235,1989.

[167] Wolf B: Reassessment of biotin-responsiveness in "unresponsive" propionylCoAcarboxylase deficiency. *J Pediatr* **97**:964,1980.

[168] Roe CR, Millington DS, Maltby DA, Bohan TP: L-carnitine enhances excretion of propionyl coenzyme A as propionylcarnitine in propionic acidemia. *J Clin Invest* **73**:1785,1984.

[169] Wolff JA, Carroll JE, Thuy LP, Prodanos C, Haas R, Nyhan WL: Carnitine reduces fasting ketogenesis in patients with disorders of propionate metabolism. *Lancet* **1**:289,1986.

[170] Roe CR, Bohan TP: L-carnitine therapy in propionic acidemia. *Lancet* **1**:1411,1982.

[171] Thompson GN, Chalmers RA, Walter JH, Bresson JL, Lyonnet SL, Reed PJ, Saudubray JM , et al.: The use of metronidazole in management of methylmalonic and propionic acidaemias. *Eur J Pediatr* **149**:792,1990.

[172] Satoh T, Narisawa K, Igarashi Y, Saitoh T, Hayasaka K, Ichinohazama Y, Onodera H, Tada K, Oohara K: Dietary therapy in two patients with vitamin B12-unresponsive methylmalonic acidemia. *Eur J Pediatr* **135**:305,1981.

[173] Morrow G, III, Burkel GM: Long-term management of a patient with vitamin B12-responsive methylmalonic acidemia. *J Pediatr* **96**:425,1980.

[174] Roe CR, Hoppel CL, Stacey TE, Chalmers RA, Tracey BM, Millington DS: Metabolic response to carnitine in methylmalonic aciduria. *Arch Dis Child* **58**:916,1983.

[175] Bain MD, Jones M, Borriello SP, Reed PJ, Tracey BM, Chalmers RA, Stacey TE: Contribution of gut bacterial metabolism to human metabolic disease. *Lancet* **1**:1078,1988.

[176] Koletzko B, Bachmann C, Wendel U: Antibiotic therapy for improvement of metabolic control in methylmalonic aciduria. *J Pediatr* **117**:99,1990.

[177] Van der Meer SB, Poggi F, Spada M, Bonnefont JP, Ogier H, Hubert P, Depondt E , et al.: Clinical outcome and long-term management of 17 patients with propionic acidaemia. *Eur J Pediatr* **155**:205,1996.

[178] Leonard JV: The management and outcome of propionic and methylmalonic acidaemia. *J Inherit Metab Dis* **18**:430,1995.

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